Serial No.: 09/497,967 Confirmation No.: 8124 Filed: February 4, 2000

For: DIAGNOSTIC AND PROTECTIVE ANTIGEN GENE SEQUENCES OF ICHTHYOPHTHIRIUS

## **Amendments to the Claims**

This listing of claims replaces all prior versions and listings of claims in the above-identified application:

- (Withdrawn currently amended) [[A]] <u>An isolated</u> nucleic acid molecule comprising a
  polynucleotide fragment having a nucleotide sequence that encodes at least a C-terminal
  portion of an i-antigen polypeptide having amino acid sequence SEQ ID NO:6.
- 2. (Withdrawn currently amended) The nucleic acid molecule of claim [[2]] 1 comprising SEQ ID NO:1.
- 3. (Currently amended) [[A]] An isolated nucleic acid molecule comprising a polynucleotide fragment having a nucleotide sequence that encodes an antigenic portion of an i-antigen polypeptide having amino acid sequence SEQ ID NO:7, said antigenic portion of the i-antigen polypeptide comprising at least about 60 amino acids and being capable of inducing an immune response in a fish against *I. multifiliis*.
- 4. (Currently amended) [[A]] An isolated nucleic acid molecule comprising a polynucleotide fragment having a nucleotide sequence that encodes at least one terminal membrane targeting portion of an i-antigen polypeptide having amino acid sequence SEQ ID NO:7, said terminal membrane targeting portion comprising at least about 10 amino acids and being capable of targeting a polypeptide to either the endoplasmic recticulum or to the plasma membrane.
- (Currently amended) [[A]] <u>An isolated</u> nucleic acid molecule comprising a
  polynucleotide fragment having a nucleic acid sequence that encodes SEQ ID NO:7.

Serial No.: 09/497,967 Confirmation No.: 8124 Filed: February 4, 2000

For: DIAGNOSTIC AND PROTECTIVE ANTIGEN GENE SEQUENCES OF ICHTHYOPHTHIRIUS

6. (Currently amended) The nucleic acid molecule of any of claims 3-5 or 36 that is a vector capable of expressing the polypeptide encoded by the nucleic acid sequence in a <u>Tetrahymena host</u> cell selected from the group consisting of a bacterium, a protozoan, a yeast, an insect cell, and an animal cell.

## 7. - 9. (Canceled)

- 10. (Currently amended) [[A]] <u>An isolated</u> nucleic acid molecule that is substantially complementary to <u>hybridizes with</u> any of the nucleic acid molecules of claims 3-5 or 36 under conditions exemplified by about 150 mM NaCl, 15 mM trisodium citrate, and pH 7.6 at 55°C.
- 11. (Currently amended) [[A]] <u>An isolated</u> nucleic acid molecule comprising a polynucleotide fragment that hybridizes to at least a portion of the complement of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:44 or SEQ ID NO:102 under standard hybridization conditions comprising about 150 mM NaCl, 15 mM trisodium citrate, and pH 7.6 at 55°C, wherein the polynucleotide fragment encodes a polypeptide comprising at least a membrane targeting portion or an antigenic portion of an i-antigen protein, wherein said antigenic portion is capable of inducing an immune response in a fish.
- 12. (Withdrawn currently amended) A polypeptide selected from the group consisting of:
  an i-antigen polypeptide having SEQ ID NO:6;
  an i-antigen polypeptide having SEQ ID NO:7;
  an analog or modification of an i-antigen polypeptide having SEQ ID NO:6;
  a fragment of an i-antigen polypeptide having SEQ ID NO:6 wherein the
  fragment comprises at least a C-terminal portion of SEQ ID NO:6;

an analog or modification of an i-antigen polypeptide having SEQ ID NO:7;

Serial No.: 09/497,967 Confirmation No.: 8124 Filed: February 4, 2000

For: DIAGNOSTIC AND PROTECTIVE ANTIGEN GENE SEQUENCES OF ICHTHYOPHTHIRIUS

a fragment of an i-antigen polypeptide having SEQ ID NO:7 wherein the fragment comprises at least at least one terminal portion of SEQ ID NO:7; and an antigenic fragment of an i-antigen polypeptide having SEQ ID NO:7; and an i-antigen polypeptide that shares a significant level of primary structure with at least one of SEQ ID NO:6 and SEQ ID NO:7.

- 13. (Withdrawn) The polypeptide of claim 12 that is antigenic.
- 14. (Currently amended) A composition for inducing an immune response in a fish, said composition comprising at least one nucleic acid molecule of any of claims [[3-7]]3-6, 10, 11 or 36.
- 15. (Withdrawn) The composition of claim 14 comprising a polypeptide of claim 13.
- 16. (Canceled)
- 17. (Original) The composition of claim 14 wherein administration of the composition to fish prevents or controls *I. multifiliis* infection.
- 18. (Previously presented) The composition of claim 14 wherein the nucleotide sequence encodes an antigenic portion of an i-antigen polypeptide linked at its carboxy-terminus to a plurality of molecules of the C3d component of complement.
- 19. (Original) The composition of claim 14 formulated for oral administration.
- 20. (Original) The composition of claim 19 wherein the polypeptide or nucleic acid molecule is encapsulated in a biodegradable polymer.

Serial No.: 09/497,967 Confirmation No.: 8124 Filed: February 4, 2000

For: DIAGNOSTIC AND PROTECTIVE ANTIGEN GENE SEQUENCES OF ICHTHYOPHTHIRIUS

- (Currently amended) A <u>Tetrahymena</u> host cell transformed with the nucleic acid molecule of claim 6.
- 22. (Withdrawn) A fish comprising the nucleic acid molecule of any of claims 1, 3 or 4.
- 23. (Currently amended) Transformed *Tetrahymena* comprising the nucleic acid molecule of any of claims [[3-11]] 3-6, 10, 11 or 36.
- 24. (Withdrawn) An antibody capable of binding a polypeptide of claim 12.
- 25. (Withdrawn) A method for detecting *Ichthyophthirius* in an aquaculture comprising: obtaining a sample containing nucleic acid from an aquaculture fish or an aquaculture water; adding at least one primer oligonucleotide having a sequence complementary to at least a portion of SEQ ID NO:1 or SEQ ID NO:3 to the nucleic acid sample; conducting a polymerase chain reaction amplification with the sample;
  - and analyzing the reaction mixture for the presence of a product amplified by the at least one oligonucleotide primer.
- 26. (Withdrawn currently amended) The method of claim 25 wherein the primer is capable of amplifying nucleotide sequences encoding i-antigens derived from at least two different *I. multifiliis* serotypes.
- 27. (Withdrawn) The method of claim 26 wherein the primer has a nucleic acid sequence that encodes an amino acid sequence selected from the group consisting of MKYNILLT (SEQ ID NO:36), FLSISLLF (SEQ ID NO:38), GTALDDGV (SEQ ID NO:46), AGTDTCT (SEQ ID NO:48), CTKKLTSGA (SEQ ID NO:50) and FAKFLSISL (SEQ ID NO:52).

oligonucleotide primer.

Serial No.: 09/497,967 Confirmation No.: 8124 Filed: February 4, 2000

For: DIAGNOSTIC AND PROTECTIVE ANTIGEN GENE SEQUENCES OF ICHTHYOPHTHIRIUS

- 28. (Withdrawn currently amended) The method of claim 25 further comprising making [[an]] a polynucleotide vaccine comprising at least a portion of the amplified product, wherein the portion of the amplified product encodes an antigenic polypeptide.
- 29. (Withdrawn) The method of claim 25 further comprising making a protein subunit vaccine comprising an antigenic polypeptide encoded by at least a portion of the amplified product.
- 30. (Withdrawn) The method of claims 28 or 29 further comprising administering the vaccine to fish to treat or prevent *Ichthyophthirius* infection.
- 31. (Withdrawn) A method for identifying an *I. multifiliis* serotype comprising:

  providing a sample comprising an *I. multifiliis* nucleic acid molecule having a nucleotide sequence encoding an i-antigen;

  adding to the sample at least one primer oligonucleotide having a sequence complementary to a unique region of an *I. multifiliis* nucleotide sequence encoding an i-antigen;

  subjecting the sample to amplification conditions; and analyzing the sample to determine the presence of a product amplified by the at least one
- 32. (Withdrawn) A method for inducing an immune response in a fish comprising administering to the fish the immunogenic composition of claim 14.
- 33. (Withdrawn) The method of claim 32 performed prophylactically in advance of exposure to *I. multifiliis*.

Serial No.: 09/497,967 Confirmation No.: 8124 Filed: February 4, 2000

For: DIAGNOSTIC AND PROTECTIVE ANTIGEN GENE SEQUENCES OF ICHTHYOPHTHIRIUS

- 34. (Withdrawn) The method of claim 32 performed therapeutically while the fish is infected with *I. multifiliis*.
- 35. (Withdrawn) The method of claim 32 wherein administration is performed by injection, immersion, or oral ingestion.
- 36. (Previously presented) The nucleic acid molecule of claim 5 comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:44 and SEQ ID NO:102.
- 37. (Canceled)

Page 7